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Abstract. The quantification of skin carotenoid levels has a range of applications in Caucasian populations, from serving as a versatile and noninvasive biomarker (e.g., of systemic carotenoid levels, carotenoid consumption, the antioxidative capacity of skin, and oxidative stress) to being used in appearance-based interventions. Yet, no study has investigated the quantitative effect of carotenoid supplementation on African skin. The aim of this study was to determine if beta-carotene supplementation produces a significant color change in three different regions of African skin. To do so we supplemented the diet of African participants with beta-carotene over an eight-week period. Reflectance spectrophotometry measurements were taken on a weekly basis for the duration of the supplementation study. Results show a significant increase in the carotenoid coloration of lightly pigmented skin (palm of the hand) and highly pigmented skin with low sun exposure (inner arm) after supplementation. The latter was no longer significant after Bonferroni correction. The carotenoid coloration of highly pigmented skin areas with high sun exposure did not increase significantly. Skin carotenoid measurements of the palm of the hand might, therefore, serve as a potential biomarker for systemic carotenoid concentrations in people of African descent. [DOI: 10.1117/1.JBO.19.2.025004]

Keywords: reflectance spectrophotometry; carotenoid; beta-carotene; yellowness; color; CIELAB b*; African.

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1 Introduction

Carotenoids form an integral part of the human antioxidant defense network, which protects the body against cellular damage caused by the harmful actions of free radicals.[1–3] Excessive amounts of free radicals can cause oxidative stress, which has been linked to a variety of negative health consequences.[4,5] Carotenoids potentially provide a protective effect against a variety of disorders linked to oxidative stress, including cardiovascular disease, certain cancers, and eye disorders,[2,6–8] although two studies found an increased incidence of lung cancer after beta-carotene supplementation in heavy smokers and individuals exposed to asbestos.[9,10] A protective effect is also consistent with the findings that low carotenoid levels were associated with increased all-cause mortality in a large study of U.S. adults.[11] Carotenoids cannot be synthesized by the human body and are obtained from the diet, primarily from red, orange, or yellow pigmented fruit and vegetables.[2,12] Plasma carotenoids are deposited in various human tissues, including the liver, adipose tissue, and skin.[13,14]

Carotenoids accumulate in human skin by either diffusing from the blood, through the hypodermis and dermis to the epidermis, or transportation to the epidermis via sweat, which contains carotenoids.[14,15] The two most prominent carotenoids in the skin are beta-carotene and lycopene.[14] Studies have shown that ultraviolet radiation[16] and infrared radiation[17] reduce the concentration of carotenoids in the skin, presumably because the carotenoids are destroyed through their interaction with radiation-induced free radicals. Oxidative stress is associated with enhanced skin aging,[18] and conversely, skin with a high carotenoid concentration appears younger[14] and has fewer furrows and wrinkles than skin with a low carotenoid concentration.[19] Supplementation with beta-carotene (and increased dietary intake of fruit and vegetables) also produces an increase in the normal skin yellowness[20–22]—but not skin redness or luminance[21]—of Caucasian skin, and studies have shown that a somewhat yellower skin color is considered healthier and more attractive by both African and Caucasian observers.[20,23–25]

Skin carotenoid concentrations are highly correlated with serum carotenoid concentrations[26–28] and can, therefore, provide a noninvasive index of systemic carotenoid concentrations. Skin carotenoid concentrations can also serve as biomarkers for fruit and vegetable consumption,[20–22,29] the antioxidative capacity of human skin, oxidative stress, in general,[30,31] and possibly vitamin A levels [since beta-carotene is a precursor of vitamin A (Ref. 32)]. Another advantage of investigating skin carotenoid concentrations is their use in appearance-based interventions.[22] Whitehead et al.[33] found that participants reported a significant, sustained increase in their fruit and vegetable consumption after researchers demonstrated the beneficial effect of fruit and vegetable consumption in a digital manipulation of the participant's own facial image. Participants who received health information and those who saw the same digital manipulation in a generic face did not report a significant increase in fruit and vegetable consumption.[33]

Very little is known about the influence of carotenoid supplementation on African skin. First, although various studies have shown an increase in skin carotenoid concentration and skin yellowness due to carotenoid supplementation in Caucasian skin,[20,21,27,28,34] to our knowledge, no study has yet

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examined the effect of carotenoid supplementation on African skin. Second, we previously found that African observers significantly increased skin yellowness (and lightness) in same race facial images to increase the facial images' apparent health.[20] It is, however, still unclear whether moderate increases in carotenoid concentrations produce perceivable yellow color differences in African skin, especially under natural sunlight conditions.

To address these gaps in the literature, this study aims to test whether beta-carotene supplementation produces a significant carotenoid-specific color change in three different regions of African skin: lightly pigmented skin, highly pigmented skin with low sun exposure, and highly pigmented skin with high sun exposure.

2 Materials and Methods

2.1 *Ethical Approval*

This study was approved in writing by the Ethics Committee at the University of Pretoria (EC110630-050). All participants gave written informed consent prior to taking part in the study.

2.2 *Participants and Study Design*

Ten black African female participants (mean age = 27.57; s.d. = 6.85) were recruited from the University of Pretoria. Participants completed a questionnaire containing questions on their age, gender, ethnicity, and the use of skin lightening products. All participants reported being of African descent and none of the participants reported using skin lightening products. The questionnaire also contained questions concerning contraindications for beta-carotene supplementation: pregnancy or breastfeeding; allergy to nuts, soya, and sulfites; or heavy smoking. According to the World Health Organization definition, a heavy smoker smokes more than 20 cigarettes daily.[35] As a conservative cutoff, we, therefore, excluded anyone who smokes more than 10 cigarettes daily. One participant was allergic to nuts and was, therefore, excluded from the study.

Using a Konica Minolta CM2600d reflectance spectrophotometer, we measured six predefined skin areas in three different regions of African skin: palm (lightly pigmented skin); inner arm (highly pigmented skin with low sun exposure); outer arm, forehead, left cheek, and right cheek (highly pigmented skin with high sun exposure). The predefined skin areas were measured in (1) CIELab color space, CIELab L* (luminance axis), CIELab a* (green-red axis), CIELab b*(blue-yellow axis), and (2) spectral reflectance values (400 to 740 nm). The measurement aperture was held lightly against the skin to minimize pressure-induced bleaching. All participants were asked to remove their makeup using hypoallergenic wipes at least 15 min before spectrophotometry measurements.

The supplementation study was conducted between August and early October 2011 (winter/early spring). Participants were provided with a week's supply of Holland & Barrett beta-carotene capsules [15 mg; containing soya bean oil, capsule shell (gelatine, glycerine), corn oil, thickener (yellow beeswax), beta-carotene, emulsifier (soya lecithin), vitamin E (as dl-Alpha tocopherol)] and asked to take one capsule daily and return on a weekly basis for 8 weeks. Each week we measured the six predefined skin areas with the spectrophotometer and participants were provided with another week's supply of beta-carotene supplements. Two participants discontinued the study in week 5 because they were experiencing symptoms that they thought might be attributed to beta-carotene supplementation (participant 1: facial rash; participant 2: nervous and dizzy), leaving seven participants for the final analysis.

2.3 *Statistical Analysis*

We performed a repeated measures general linear model (GLM), with measurement area (forehead, left cheek, right cheek, inner arm, outer arm, and palm) and week (weeks 0 to 8) as within-subject factors. Next, separate repeated measures GLMs were performed for each measurement area to determine which measurement areas showed significant increases in skin yellowness. To control for multiple testing, we adjusted the alpha level to a conservative $\alpha = 0.005$ using Bonferroni correction (0.05/10). The difference in spectral reflectance values before and after supplementation were used to verify whether the change in CIELab b* values was most likely due to carotenoids. All analyses were performed in SPSS version 21.

3 Results

3.1 *Descriptive Statistics*

A descriptive table with CIELab b*, CIELab L*, and CIELab a* values before and after supplementation are provided in Table 1.

3.2 *Repeated Measures GLM*

Muachly's test indicated that the assumption of sphericity had been somewhat violated for measurement area [$x^2(14) = 25.84$, $p = 0.049$); therefore, degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ($\varepsilon = 0.42$). The repeated measures GLM analysis of CIELab b* revealed a significant main effect of measurement area [$F(2, 30) = 5.530$, $p = 0.018$, $\eta p^2_{\text{partial}} = 0.480$, observed power = 0.768] and week [$F(8, 48) = 2.375$, $p = 0.031$, $\eta p^2_{\text{partial}} = 0.284$, observed power = 0.836]. There was also a significant interaction between week and measurement area [$F(40,240) = 2.235$, $p \leq 0.001$, $\eta p^2_{\text{partial}} = 0.271$, observed power = 1.000]. These results indicate that there was a difference in skin yellowness between the different measurement areas (Fig. 1). More importantly, results show that there was an increase in participants' skin yellowness over the 8-week supplementation study (CIELab b* before: mean = 18.856, s.d. = 1.807; CIELab b* after: mean = 19.165, s.d. = 2.418), with certain measurement areas showing a larger change in CIELab b* than others (Fig. 1). The interaction between week and measurement area (but neither of the main effects) was still significant at the conservative Bonferroni adjusted level of $\alpha = 0.005$.

Separate repeated measures GLMs for each measurement area were used to determine which measurement areas showed significant increases in skin yellowness. When analyzed separately, only the palm [$F(8, 48) = 4.324$, $p = 0.001$, $\eta p^2_{\text{partial}} = 0.419$, observed power = 0.988] and inner arm [$F(8, 48) = 3.002$, $p = 0.008$, $\eta p^2_{\text{partial}} = 0.333$, observed power = 0.923] measurement areas showed a significant increase in skin yellowness over the 8-week supplementation study (all other measurement areas $p \geq 0.11$; Fig. 1). The CIELab b* increase in the palm remained significant at Bonferroni adjusted alpha, but the CIELab b* increase in the inner arm did not. Since CIELab a* (skin redness) values in the palm of the hand also increased substantially after

Table 1 Mean CIELab b*, CIELab L*, and CIELab a* values at the different measurement areas before and after supplementation. Standard deviations are indicated in brackets.

	CIELab b*			CIELab L*			CIELab a*		
	Before	After	Change	Before	After	Change	Before	After	Change
Palm	17.66 (1.58)	19.33 (2.41)	1.67	58.30 (1.88)	58.30 (0.95)	0.00	10.13 (1.40)	12.78 (1.49)	2.65
Inner arm	18.64 (1.55)	19.78 (1.72)	1.14	51.50 (4.79)	49.98 (5.06)	−1.52	8.64 (0.76)	8.99 (0.83)	0.35
Outer arm	18.18 (3.00)	18.42 (3.14)	0.24	45.23 (6.17)	43.83 (6.00)	−1.40	10.53 (0.57)	10.54 (0.78)	0.01
Forehead	17.49 (3.36)	17.16 (4.10)	−0.33	43.43 (5.27)	42.63 (5.28)	−0.80	11.91 (0.84)	11.67 (1.19)	−0.24
Left cheek	20.45 (1.40)	20.01 (2.41)	−0.44	48.42 (4.29)	47.22 (5.04)	−1.20	11.50 (0.77)	11.41 (0.78)	−0.09
Right cheek	20.71 (2.00)	20.29 (2.21)	−0.42	48.26 (4.71)	47.17 (4.04)	−1.09	11.32 (0.66)	11.46 (0.86)	0.14

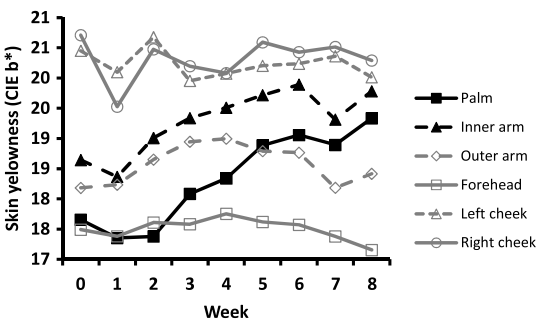

Fig. 1 The change in skin yellowness (CIELab b* values) over the 8-week supplementation study for different measurement areas. The palm (solid square) and the inner arm (solid triangle) were the only two measurement areas that showed a significant increase in CIELab b* values.

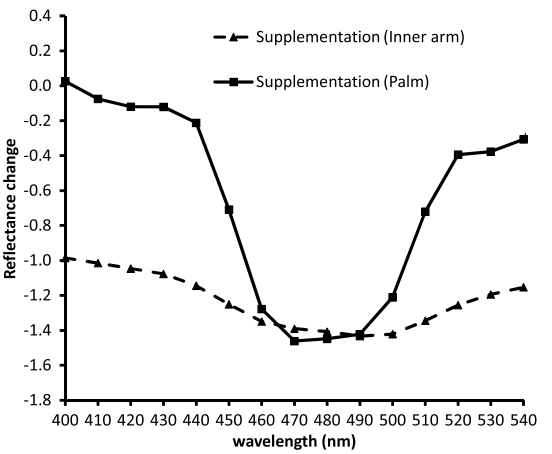

Fig. 2 Change in spectral reflectance values due to dietary supplementation of beta-carotene. The graph shows a trough at ~450 to 490 nm for both measurements (especially the palm), which is consistent with an increase in light adsorption by skin carotenoid pigment.

supplementation (Table 1) and since basal CIELab b* and CIELab a* are highly correlated in the palm of African skin ($r_s = 0.786$, $p = 0.036$), we performed a repeated measures GLM of CIELAb a* in the palm of the hand to compare the variance explained by CIELab b* and CIELab a*. The palm measurement area also showed a significant increase in skin redness over the 8-week supplementation study [$F(8, 48) = 3.419$, $p = 0.003$, $\eta p^2_{\text{partial}} = 0.363$, observed power = 0.956], but CIELab a* explained less variance than CIELAb b* ($\eta p^2_{\text{partial}} = 0.419$), indicating that CIELab b* is driving the association between beta-carotene supplementation and skin color change.

3.3 *Change in Spectral Reflectance due to Dietary Supplementation of Beta-Carotene*

To verify whether the change in CIELab b* was likely to be due to a change in carotenoid coloration, we calculated the change in spectral reflectance values across the supplementation study for the palm and inner arm (week 8 to week 0). The differences were then averaged across all participants for each position (Fig. 2). Lower values indicate that a smaller proportion of incident light is reflected (which is consistent with a greater light adsorption by skin pigments). Based on the literature, we expected a decrease in spectral reflectance values at 450 to 490 nm—the wavelengths associated with peak light absorption by beta-carotene[20,36]—if skin carotenoid coloration increased across the supplementation study. As expected, we observed a decrease in spectral reflectance values at ~450 to 490 nm (especially for palm measurements), indicating that the change in CIELab b* values were likely to be due to a change in carotenoid coloration (Fig. 2).

4 Discussion

The aim of this study was to determine whether beta-carotene supplementation produces a significant yellow color change in different regions of African skin. To our knowledge, this is the first study to test the effect of carotenoid supplementation in African skin. Our results show that oral supplementation with a daily dose of 15 mg beta-carotene over the course of 8 weeks was associated with a significant increase in skin yellowness in African skin, primarily in lightly pigmented skin (e.g., palm), but also to some extend in highly pigmented skin with low sun exposure (e.g., inner arm). Consistent with previous findings in Caucasian skin,[20] beta-carotene supplementation was

most strongly associated with CIELab b* skin color changes in the palm measurement area (explaining 42% of the variance). CIELab a* values also increased significantly in the palm measurement area across the supplementation study. This is not unexpected, given the high correlation between CIELab b* and CIELab a* values in African skin.[24,25] Our results show that CIELab b* is, however, driving the association between beta-carotene supplementation and skin color change. Palm measurements of CIELab b* levels might, therefore, serve as a potential marker for systemic carotenoid concentrations;[26,27] fruit and vegetable consumption;[20–22,29] the antioxidative capacity of human skin; oxidative stress, in general;[30,31] and possibly vitamin A levels in the African population [since beta-carotene is a precursor of vitamin A (Ref. 32)].

We should point out some limitations to the study. First, the study had a fairly low sample size. Nevertheless, repeated measures substantially increase the power of the analysis (e.g., Ref. 37), and *post hoc* power analyses indicate that the study had sufficient power (observed power > 0.8) to detect a beta-carotene supplementation effect on skin coloration. Second, we did not include a washout period before and after the supplementation study, which might have increased the noise in beta-carotene levels. Third, we did not measure plasma carotenoid levels directly. It is, however, highly likely that the plasma carotenoid levels increased after supplementation, given that we observed a significant increase in carotenoid-specific coloration in the palm of the hand after beta-carotene supplementation. Nevertheless, future studies could benefit from including plasma carotenoid levels, washout periods, and a larger sample of male and female participants.

We did not find a significant increase in skin yellowness in highly pigmented skin that has high sun exposure (e.g., outer arm and face). This is most likely because an increase in melanin pigmentation due to increased sun exposure in early spring—as evidenced by decreased CIELab L* values (Table 1)—overshadowed the changes in carotenoid pigmentation. Another nonmutually exclusive explanation is that carotenoids accumulated during the supplementation study may have been oxidized through increased ultraviolet and infrared exposure during early spring. It is, therefore, unlikely that participants will be able to observe a beneficial effect of fruit and vegetable consumption in sun-exposed highly pigmented African skin under natural sunlight conditions. Of course, a change in carotenoid coloration might also be observable in sun-exposed highly pigmented African skin during late summer/early winter or if participants consume more fruit and vegetables or higher doses of carotenoid supplementation.

In summary, we found a significant increase in skin yellowness of African skin after an 8-week beta-carotene supplementation study, but only in lightly pigmented skin (e.g., palm) and to some extend in highly pigmented skin with low sun exposure (e.g., inner arm). Skin carotenoid measurements in the palm of the hand or sun-protected skin areas might, therefore, serve as a potential marker for systemic carotenoid concentrations in people of African descent.

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